

## INVITED REVIEW

# Ecophysiology of Crassulacean Acid Metabolism (CAM)

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- **Background and Scope** Crassulacean Acid Metabolism (CAM) as an ecophysiological modification of photosynthetic carbon acquisition has been reviewed extensively before. Cell biology, enzymology and the flow of carbon along various pathways and through various cellular compartments have been well documented and discussed. The present attempt at reviewing CAM once again tries to use a different approach, considering a wide range of inputs, receivers and outputs.
- **Input** Input is given by a network of environmental parameters. Six major ones, CO<sub>2</sub>, H<sub>2</sub>O, light, temperature, nutrients and salinity, are considered in detail, which allows discussion of the effects of these factors, and combinations thereof, at the individual plant level ('physiological aut-ecology').
- **Receivers** Receivers of the environmental cues are the plant types genotypes and phenotypes, the latter including morphotypes and physiotypes. CAM genotypes largely remain 'black boxes', and research endeavours of genomics, producing mutants and following molecular phylogeny, are just beginning. There is no special development of CAM morphotypes except for a strong tendency for leaf or stem succulence with large cells with big vacuoles and often, but not always, special water storage tissues. Various CAM physiotypes with differing degrees of CAM expression are well characterized.
- **Output** Output is the shaping of habitats, ecosystems and communities by CAM. A number of systems are briefly surveyed, namely aquatic systems, deserts, salinas, savannas, restingas, various types of forests, inselbergs and paramós.
- **Conclusions** While quantitative census data for CAM diversity and biomass are largely missing, intuition suggests that the larger CAM domains are those systems which are governed by a network of interacting stress factors requiring versatile responses and not systems where a single stress factor strongly prevails. CAM is noted to be a strategy for variable, flexible and plastic niche occupation rather than lush productivity. 'Physiological syn-ecology' reveals that phenotypic plasticity constitutes the ecophysiological advantage of CAM.

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**Key words:** CAM, ecophysiology.

## INTRODUCTION

The first comprehensive review of Crassulacean Acid Metabolism (CAM) was published in 1960 (Wolf, 1960). Subsequently, CAM research has developed continuously and extensively, and has been reviewed widely in articles and books (Kluge and Ting, 1978; Osmond, 1978; Kluge, 1979; Queiroz, 1979; Cockburn, 1985; Ting, 1985; Winter, 1985; Lüttge, 1987, 1989a, 1993, 1998, 2002b, 2003a; Griffiths, 1988a; Winter and Smith, 1996a; Cushman and Bohnert, 1997, 1999; Cushman, 2001; Cushman and Borland, 2002; Dodd *et al.*, 2002; Functional Plant Biology, 2002). This covers aspects of CAM ranging from ecosystems and ecology, physiology and metabolic pathways, cell biology, and transport and compartmentation to molecular biology.

The simplest definition of CAM, first described for species of the family Crassulaceae, is that there is (1) nocturnal uptake of CO<sub>2</sub> via open stomata, fixation by phosphoenolpyruvate carboxylase (PEPC) and vacuolar storage of CO<sub>2</sub> in the form of organic acids, mainly malic acid (*phase I sensu* Osmond, 1978), and (2) daytime remobilization of vacuolar organic acids, decarboxylation

and refixation plus assimilation of CO<sub>2</sub> behind closed stomata in the Calvin-cycle (*phase III*). Between these two phases there are transitions when stomata remain open for CO<sub>2</sub> uptake for a short time during the very early light period (*phase II*) and reopen again during the late light period for CO<sub>2</sub> uptake with direct assimilation to carbohydrate when vacuolar organic acid is exhausted (*phase IV*).

Phases II and IV respond very sensitively to environmental input parameters. However, versatility is greater than flexibility of expression of CAM phases. This characteristic has affected CAM terminology and definitions. Cockburn (1985) has made several fine distinctions, which are interesting as they distinguish between the performance of plants and plant organs with and without stomata, and also between terrestrial and aquatic plants. The following two distinctions are most important. First, CAM idling (Sipes and Ting, 1985), where stomata remain closed day and night and the day/night organic acid cycle is fed by internal recycling of nocturnally refixed respiratory CO<sub>2</sub>. Much has been written on this phenomenon of CAM in response to severe stress due to limitations of water availability (Griffiths, 1988b, 1989; Griffiths *et al.*, 1989). Secondly, CAM cycling (Sipes and Ting, 1985), where stomata remain closed during the dark period but some

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nocturnal synthesis of organic acid fed by respiratory CO<sub>2</sub> occurs, and where stomata are open during the light period with uptake of atmospheric CO<sub>2</sub> and direct Calvin-cycle CO<sub>2</sub> reduction (C<sub>3</sub>-photosynthesis) in addition to assimilation of CO<sub>2</sub> remobilized from nocturnally stored organic acid.

CAM idling is considered as a form of very strong CAM, while CAM cycling is weak CAM. In the epiphytic Gesneriaceae *Codonanthe crassifolia*, Guralnick *et al.* (1986) observed CAM cycling in well-watered plants and CAM idling in drought-stressed plants. CAM cycling that scavenges respiratory CO<sub>2</sub> appears to be a sort of prelude to 'real' CAM, and this work, as well as studies on the Portulacaceae (Guralnick and Jackson, 2001), suggests that it might have been a starting point for CAM evolution. Thus, the various forms of weak and strong CAM may be restricted to different individual species (Guralnick and Jackson, 2001), which then constitute different CAM physiotypes. However, they may also be expressed temporarily in one given species. For example, *Sedum telephium* has the potential to exhibit pure C<sub>3</sub> characteristics when well-watered and a transition to CAM when droughted, including a continuum of different stages of CAM expression which are repeatedly reversible under changing drought and watering regimes (Lee and Griffiths, 1987).

Cockburn (1998) speculates that cycling of CO<sub>2</sub> via malate into the Calvin cycle may also occur at higher than diurnal frequencies, and this 'rapid-cycling CAM' will be limited to daytime. However, this is difficult to distinguish from one-cell C<sub>4</sub> photosynthesis that does not require the different cell types of mesophyll and bundle-sheath (Magnin *et al.*, 1997; Freitag and Stichler, 2000, 2002; Akhani *et al.*, 2003) and also from the function of stomatal guard cells that, in Cockburn's view, are also close to performing CAM. Stomatal guard cells use malate synthesis by PEPC and vacuolar storage of potassium malate as osmoticum in turgor-driven opening movements and remobilize the malate during the closing movements. Photosynthetic carbon flow in some brown algae, where malate serves as an intermediate store of fixed CO<sub>2</sub>, has similarities to both CAM and C<sub>4</sub> photosynthesis (Raven *et al.*, 1985; Schmid and Dring, 1996; Keeley, 1996; Schmid *et al.*, 1996). In higher plants, during nitrate reduction in the leaves, malic acid is synthesized by PEPC where the protons of the acid are used to neutralize the hydroxyl ions produced by nitrate reduction, and the malate anion is stored as its potassium salt in the vacuole during the day and remobilized again during the night (Winter *et al.*, 1982a; Gerhardt and Heldt, 1984; see Lüttege and Clarkson, 1987). Thus, there are observed both acid fluctuations without malate fluctuations and malate fluctuations without acid fluctuations, neither of which could be called CAM. However, the case for CAM in some submerged freshwater plants is intriguing. In these plants, CO<sub>2</sub> for dark fixation by PEPC is not taken up by the leaves but is supplied via the roots, protons of malic acid synthesized are exchanged for K<sup>+</sup>, and there are CAM-type malate oscillations without acid oscillations (Raven *et al.*, 1988; Cockburn, 1998).

Hence, from all the above, it is apparent that a coherent and comprehensive definition of CAM is not

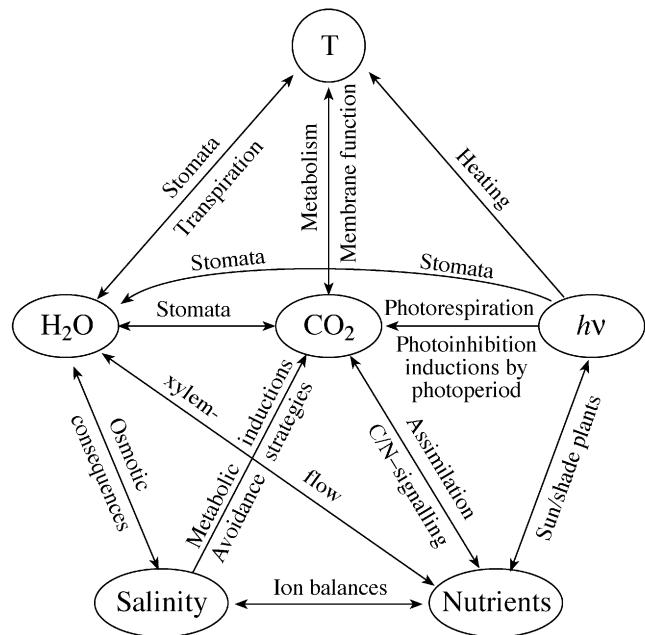


FIG. 1. Network of the most important environmental parameters and connections of their effects in CAM.

straightforward (Holtum, 2002). The high versatility of CAM and CAM-like behaviour alluded to is certainly also related to the fact that there is nothing specific in the enzymatic complement of CAM. Although there are certainly CAM-specific isoforms of some of the enzymes involved, there are basically no CAM-typical enzymes (Lüttege, 1998, 2003a).

## INPUT

### *Environmental parameter network*

In ecophysiology principally we need to consider any possible environmental input to the organisms. Even if we only take the six environmental parameters which have been recognized as most important for CAM and which are studied most intensely, i.e. CO<sub>2</sub>, water, light, temperature, salinity and nutrients, and summarize their most prominent interactions we already arrive at a complex network. This is depicted in Figure 1, which represents a closed network where all factors are directly or indirectly connected and feeding back on each other. The major connections are listed in Table 1. This network structure should be kept in mind as I assess the ecophysiological impacts of the individual factors one by one and highlight some of their interactions.

### *Carbon dioxide (CO<sub>2</sub>)*

Generally, water is considered to be the most important factor and CAM to be an adaptation to water-shortage stress because transpirational water loss is minimized by CO<sub>2</sub> acquisition via open stomata during the dark period and CO<sub>2</sub> assimilation behind closed stomata during the light period.

TABLE 1. Most important connections in the network of factor functions in CAM

First factor	Second factor	Mode of coupling
CO <sub>2</sub>	H <sub>2</sub> O	Tight coupling via stomatal regulation
	Light	Multiple interactions as light is the energy source of photosynthetic CO <sub>2</sub> -assimilation and also drives photorespiration, affects photoinhibition and, via signalling pathways, may elicit CAM-induction in some plant species
	Temperature	Due to effects on metabolism and membrane functions (compartmentation) in the carbon flow of CAM
	Salinity	Salt-stress elicited CAM induction
	Nutrients	Importance for the maintenance of the photosynthetic apparatus and in carbon/nitrogen signalling systems in plant metabolism
H <sub>2</sub> O	CO <sub>2</sub>	As above
	Light	Via effects on stomata
	Temperature	Via effects on stomata and transpiration
	Salinity	Due to osmotic consequences
	Nutrients	Due to the importance of xylem flow for both water and nutrient supply in the whole plant
Light	CO <sub>2</sub>	As above
	H <sub>2</sub> O	As above
	Temperature	Via heating the plants
	Nutrients	Via the importance of nitrogen
Temperature	CO <sub>2</sub>	As above
	H <sub>2</sub> O	As above
	Light	As above
Salinity	CO <sub>2</sub>	As above
	H <sub>2</sub> O	As above
	Nutrients	Via affecting ion balances
Nutrients	CO <sub>2</sub>	As above
	H <sub>2</sub> O	As above
	Light	As above
	Salinity	As above

The six factors shown in Fig. 1 represent the ‘first factors’ and their connections to the other factors are represented by the ‘second factors’.

However, CAM is also observed in submerged freshwater plants (Keeley, 1996), where CO<sub>2</sub> fixation via PEPC with its high affinity to its HCO<sub>3</sub><sup>-</sup> substrate and in the absence of competition from C<sub>3</sub>-photosynthetic organisms during the dark period sustains an internal CO<sub>2</sub>-concentrating mechanism (Griffiths, 1989; Lüttge, 2002b). As well as some angiosperms, such submerged plants also include species of *Isoëtes*, a much more basic taxon of vascular plants’ phylogeny. CO<sub>2</sub> may, therefore, be considered as the central factor and most important driving force for the earliest evolution of CAM (Griffiths, 1989). Thus, it might be assumed that early CAM evolution in *Isoëtes* occurred during geological times when atmospheric CO<sub>2</sub> concentration,  $p^a_{CO_2}$ , was low. Indeed, the early evolution of the Isoëtales during the Permian, about 250 × 10<sup>6</sup> years ago, coincided well with a time of decreasing  $p^a_{CO_2}$  during the Phanerozoic (see Berner, 1994; Laws *et al.*, 2002). However, among more basic taxa, CAM is also frequently found in terrestrial, i.e. epiphytic and lithophytic, ferns (Ong *et al.*, 1986; Winter *et al.*, 1986; Holtum and Winter, 1999; Sayed, 2001). With its multifactor responses, and the fact that no special enzymes are required and a well-managed general enzyme complement suffices, CAM clearly evolved polyphyletically many times in the plant kingdom (see Lüttge, 2003b). Various facets of possible driving forces for the evolution of CAM have been considered by Raven and Spicer (1996).

When considering CO<sub>2</sub>, it is important to distinguish between atmospheric partial pressure,  $p^a_{CO_2}$ , the environmental factor *sensu stricto*, and internal partial pressure,

$p^i_{CO_2}$ , which are closely related to each other via stomatal opening and closing.

Above all else, CAM is a CO<sub>2</sub>-concentrating mechanism (for reviews see Griffiths, 1989; Lüttge, 2002b). This is due to the much higher substrate affinity of PEPC for HCO<sub>3</sub><sup>-</sup> than of the C<sub>3</sub>-photosynthesis/Calvin cycle carboxylase Rubisco(ribulose-bis-phosphate carboxylase/oxygenase) for CO<sub>2</sub>. Thus, during the dark period a concentrated CO<sub>2</sub> pool is built up in the form of vacuolar malic acid accumulation, and during phase III its remobilization in the light leads to internal CO<sub>2</sub> concentrations that may be 2–60 times  $p^a_{CO_2}$  (Table 2). For aquatic plants, this CO<sub>2</sub>-concentrating mechanism provides a benefit for CO<sub>2</sub> acquisition. For terrestrial plants, the benefit of CO<sub>2</sub>-concentrating by CAM is considered to be related to water use, and will be discussed below.

If internal CO<sub>2</sub>-concentrating of CAM is a benefit at low  $p^a_{CO_2}$ , it might be expected that the current man-made increase in  $p^a_{CO_2}$  attenuates this advantage of CAM. However, most studies performed so far indicate a growth stimulation of CAM plants by elevated CO<sub>2</sub> concentrations (Drennan and Nobel, 2000). Stimulation is related to phytohormone action (Li *et al.*, 2002). In an orchid plantlet obtained from tissue culture, 1 % CO<sub>2</sub> stimulated growth although PEPC and Rubisco levels were reduced (Gouk *et al.*, 1997). Increased  $p^a_{CO_2}$  compensated for the inhibitory effect of increased temperature on nocturnal CO<sub>2</sub> fixation (Zhu *et al.*, 1999). An induction of CAM in the C<sub>3</sub>/CAM-intermediate species *Portulacaria afra* was not mediated by alteration of CO<sub>2</sub> availability (Huerta and Ting, 1988). A

TABLE 2. Maximum internal  $\text{CO}_2$ -concentrations ( $p^i_{\text{CO}_2}$ ),  $c_i/c_a$ -ratios (where  $c_a$  was taken as 0.04 %) and ratios of internal concentrations of  $\text{O}_2 : \text{CO}_2$  (ambient air  $\text{O}_2 : \text{CO}_2 = 633$ ) during the light period at midday, in Phase III of CAM

Species	Family	$p^i_{\text{CO}_2}$ (%)	$c_i/c_a$	$\text{O}_2 : \text{CO}_2$
<i>Opuntia ficus-indica</i>	Cactaceae	1.30	32.5	
<i>Opuntia basilaris</i>	Cactaceae	2.50	62.5	
<i>Opuntia monacantha</i>	Cactaceae	0.12	3.0	205
<i>Agave desertii</i>	Agavaceae	0.80	20.0	
<i>Yucca schidigera</i>	Agavaceae	0.40	10.0	
<i>Aloë vera</i>	Liliaceae	0.60	15.0	
<i>Ananas comosus</i>	Bromeliaceae	0.50	12.5	
<i>Ananas comosus</i>	Bromeliaceae	0.13	3.3	161
<i>Cattleya</i> sp.	Orchidaceae	0.15	3.8	
<i>Phalaenopsis</i> sp.	Orchidaceae	0.23	5.8	
<i>Hoya carnosa</i>	Asclepiadaceae	0.08	2.0	285
<i>Huernia</i> sp.	Asclepiadaceae	0.14	3.5	174
<i>Kalanchoë gastonis-bonnieri</i>	Crassulaceae	0.27	6.8	155
<i>Kalanchoë tomentosa</i>	Crassulaceae	0.35	8.8	88
<i>Kalanchoë daigremontiana</i>	Crassulaceae	0.50	12.5	
<i>Kalanchoë tubiflora</i>	Crassulaceae	0.09	2.3	
<i>Sedum praealtum</i>	Crassulaceae	0.29	7.3	81
<i>Sedum morganianum</i>	Crassulaceae	0.26	6.5	
<i>Sempervivum tectorum</i>	Crassulaceae	0.12	3.0	

From Lüttge, 2002b and references therein.

doubling of  $p^a_{\text{CO}_2}$  from 370 to 750 p.p.m. had no significant effects in the CAM plant *Agave vilmoriniana* (Szarek *et al.*, 1987). Doubling  $p^a_{\text{CO}_2}$  increased the productivity of *Opuntia ficus-indica* by 35 % on average, mostly as a result of increases in both night-time and daytime  $\text{CO}_2$  uptake, while in *Kalanchoë pinnata* increased  $p^a_{\text{CO}_2}$  mainly increased phase IV  $\text{CO}_2$  uptake (Winter *et al.*, 1997). The responses of CAM plants to increased  $p^a_{\text{CO}_2}$  are similar to those predicted for  $\text{C}_3$  plants, but much greater than those for  $\text{C}_4$  plants. There appears to be no expectation of downward acclimation (Wang and Nobel, 1996). Of course, the time scales of these observations are much too short to make any predictions on future changes. Overall, on a percentage basis effects are very small and in detail there are many uncertainties (Poorter and Navas, 2003). Currently, with the time-scales of ecosystem responses given, ecologically noticeable changes in habitat occupation by CAM plants may not be expected.

The consequences of elevated  $p^i_{\text{CO}_2}$ , i.e.  $\text{CO}_2$ -concentrating, have been reviewed recently (Lüttge, 2002b). They mainly pertain to photorespiration, photoinhibition and oxidative stress. Earlier suggestions that these are minimized by  $\text{CO}_2$  concentrating in the internal air spaces of CAM organs in phase III to the extent shown in Table 2 are based on the expectation that Rubisco begins to become substrate saturated above 0.1 %  $\text{CO}_2$  (Berry and Downton, 1982). Thus, high  $p^i_{\text{CO}_2}$  would suppress the oxygenase activity of Rubisco and hence photorespiration in the  $\text{CO}_2/\text{O}_2$  substrate competition at Rubisco. The high photochemical work of saturated  $\text{CO}_2$  assimilation would prevent over-energization of the photosynthetic apparatus, and hence suppress photoinhibition and oxidative stress. However, observations contradict these expectations. Photorespiration, photoinhibition and oxidative stress in the light occur in CAM plants

not only in phase IV, when they operate like  $\text{C}_3$  plants, but also in phase III with highly elevated  $p^i_{\text{CO}_2}$ . Substrate-saturated photosynthetic  $\text{CO}_2$  reduction behind closed stomata leads to elevated internal oxygen concentrations (Table 2), e.g. up to approx. 40 % in the CAM plant *Kalanchoë gastonis-bonnieri* (Spalding *et al.*, 1979). This high  $p^i_{\text{O}_2}$ , as a consequence of high  $p^i_{\text{CO}_2}$ , supports the formation of reactive oxygen species. In fact, CAM plants have developed very effective antioxidative response systems (Castillo, 1996; Miszalski *et al.*, 1998; Broetto *et al.*, 2002).

Another function of  $p^i_{\text{CO}_2}$  is signalling, and of particular ecophysiological relevance is regulation of stomatal opening. Increasing  $p^i_{\text{CO}_2}$  affects stomatal closure. In general, the  $\text{CO}_2$  response sensitivity of guard cells is very variable between species and also for a given species due to acclimation (Frechilla *et al.*, 2002). There appear to be no data on the critical level of  $p^i_{\text{CO}_2}$  needed to close stomata in phase III of CAM. Apparently, stomata of CAM plants have a similar  $\text{CO}_2$  sensitivity to those of  $\text{C}_3$  plants, particularly in the range of 0–36 Pa, and a half-saturation constant of 19.6 Pa was observed for both  $\text{C}_3$  and CAM plants (Jewer *et al.*, 1985). The internal  $\text{CO}_2$  concentrations in phase III are usually much higher and generally it is agreed that they are the most relevant cause of stomatal closure during daytime in this phase. Another interesting effect of  $p^i_{\text{CO}_2}$ -signalling is synchronization of the photosynthetic activities of individual cells or cell patches in leaves of CAM plants, e.g. during free-running circadian oscillations of CAM (Rascher *et al.*, 2001; Rascher and Lüttge, 2002; Lüttge, 2002b).

#### Water ( $\text{H}_2\text{O}$ )

For terrestrial plants, the greatest benefit of CAM is considered to be increased water use efficiency (WUE) because stomatal opening during the dark period causes much less transpirational loss of water than opening during the light period. Indeed, the WUE of mol  $\text{CO}_2$  fixed : mol  $\text{H}_2\text{O}$  transpired during night-time  $\text{CO}_2$  acquisition in CAM is estimated as about  $6-30 \times 10^{-3}$ . Conversely,  $\text{C}_3$  plants have only  $0.6-1.3 \times 10^{-3}$  and  $\text{C}_4$  plants  $1.7-2.4 \times 10^{-3}$ ; but for phase IV of CAM, WUEs are similarly low, i.e.  $1-4 \times 10^{-3}$  (Black, 1973). Thus, WUE is related to CAM phases. That this is ecologically important is also seen in the fact that daytime phases II and IV are suppressed under the influence of drought stress, both in the laboratory (Smith and Lüttge, 1985; Lüttge, 1987) and in the field (Lee *et al.*, 1989).

With this high WUE CAM plants might typically be expected to inhabit arid habitats. However, although CAM plants, such as cacti, agaves and euphorbs, often determine the physiognomy of deserts, CAM species inhabiting tropical rainforests far outnumber typical desert species (Table 3). Many of these species are epiphytic and subject to the particular problems of water supply in this habitat (Zotz and Hietz, 2001). It is estimated that approx. 57 % of all epiphytes are CAM plants. Therefore, high WUE might be the major ecophysiological trait of CAM and the foremost driving force of CAM evolution (Gil, 1986). However, Eller

TABLE 3. Relevance of CAM among major plant families of rainforests and deserts, where it is assumed that almost all Cactaceae and Agavaceae species and half of all Orchidaceae and Bromeliaceae species are CAM species

Major families	Number of species
Desert succulents	
Cactaceae	1500
Agavaceae	300
Total number of species	1800
CAM species	1800
Rainforest species	
Orchidaceae	19 000
Bromeliaceae	2500
Total number of species	21 500
CAM species	10 700

and Ferrari (1997) found that the C<sub>3</sub> plant *Othonna opima* (Asteraceae) and the CAM plant *Cotyledon orbiculata* (Crassulaceae) with morphologically very similar succulent life-forms showed similar WUE under the same environmental conditions. Moreover, Pierce et al (2002) have even unravelled competitive abilities of CAM bromeliads of the genus *Aechmea* in an extremely wet tropical cloud forest. During the wet season, when rainfall, mist and wetting of leaf surfaces inhibit gas exchange, total CO<sub>2</sub> uptake is superior in the CAM bromeliads compared with that of C<sub>3</sub> bromeliads, where gas exchange is limited to the light period. During the dry season, the water-saving properties are to the advantage of CAM bromeliads. It is in particular the flexibility of CAM in contrast to C<sub>3</sub> photosynthesis that supports the observed recent radiation of this photosynthetic pathway into cloud forests (Pierce et al., 2002). Based on comparative morphology of roots, of tank formation and of solute absorbing epidermal scales, Smith (1989) concluded that the common ancestor of extant epiphytic bromeliads must have been a pre-adapted, drought-tolerant terrestrial C<sub>3</sub> type and that epiphytism and CAM evolved several times and independent of each other in the Bromeliaceae.

In addition to CAM phase-dependent stomatal responses affecting WUE, CAM plants have other structural and functional ways of dealing with the water factor that are involved in short-, medium- and long-term storage of water.

Short-term effects are due to diurnal cycles of osmotic relations. The large vacuolar concentrations of nocturnally accumulated organic acids are osmotically active. The increased osmotic pressure ( $\pi$ ) drives water uptake into the cells, which is associated with increased turgor pressure ( $P$ ). This allows CAM plants extra acquisition of water, particularly towards the end of the dark period when vacuolar organic acid levels become rather high. It may be a particular advantage in moist, tropical forests with dew formation occurring mainly during the late dark period. During acid remobilization in phase III,  $P$  and  $\pi$  decline again but the water gained is available to the plants (Lüttge, 1986; Eller and Ruess, 1986; Ruess et al., 1988; Eller et al., 1992; Murphy and Smith, 1998).

Special features of water transport across membranes are also involved in the cellular water storage of CAM plant

cells. Ohshima et al. (2001) reported that the content of aquaporins, and hence water permeability, in the plasma-lemma and tonoplast of leaf cells in some CAM plants is very low. Aquaporins have also been studied in the annual C<sub>3</sub>/CAM intermediate *Mesembryanthemum crystallinum* (Yamada et al., 1995; Kirch et al., 2000; Yamada and Bohnert, 2000), where a decrease in hydraulic conductivity of cell membranes has been shown upon CAM induction (Rygal et al., 1989; Trofimova et al., 2003).

Medium-term water storage occurs where CAM plants form external water reservoirs (phytotelmata), especially the tanks typical of many bromeliads. This allows, for example, water obtained from small occasional rainfalls, which can occur even during dry periods in seasonal precipitation regimes, to be stored for several days (Lee et al., 1989).

More long-term water storage is possible due to special non-green and non-photosynthetical water storage tissues, i.e. hydrenchymas. These may be peripheral tissues such as the large epidermal bladders of the annual C<sub>3</sub>/CAM intermediate species *M. crystallinum* (Haberlandt, 1904) and the epidermal and subepidermal layers of hydrenchyma cells of leaves of *Peperomia* and bromeliads (Gibeaut and Thomson, 1989; Lee et al., 1989; Horres and Zizka, 1995). Central water storage tissues are typical of leaf-succulent agaves and many stem-succulent CAM taxa.

The biophysical basis of such water storage and water remobilization as required under stress, i.e. cellular water transport, membrane hydraulic conductivities ( $L_p$ ) and osmotic relations ( $\pi$ ,  $P$  and the cell wall elastic modulus,  $\epsilon$ ), have been studied extensively in *M. crystallinum* (Steudle et al., 1975, 1977) and in *Agave deserti* (Smith and Nobel, 1986, Smith et al., 1987). Under extended rainless periods, water can be remobilized from the water storage tissues to protect metabolically active tissue for many weeks (Schulte and Nobel, 1989). Water may also be transported from older to younger leaves (Donatz and Eller, 1993; Tüffers et al., 1995). In desert cacti and agaves, the reliance on internal water reserves may be coupled to CAM idling, so that there is no water loss by stomatal transpiration and only water loss by cuticular transpiration needs to be compensated for (Szarek and Ting, 1975; Holthe and Szarek, 1985; Lüttge et al., 1989). However, when the plants lose more than 50 % of their total water they die. Thus, regular seasonal rains are required to refill the reserves (Holthe and Szarek, 1985). For *Peperomia magnoliaefolia* this has been specified for different tissues. Entire leaves can lose up to 50 % of their total water, the water storing hydrenchyma can lose 75–85 %, but the photosynthetically active chlorenchyma suffers when it loses just 15–25 % (Schmidt and Kaiser, 1987).

The performance of the peripheral water storage tissue of epidermal bladders of *M. crystallinum* in relation with the diurnal osmotic oscillations given by malate accumulation and remobilization in the mesophyll has been analysed by Rygal et al. (1986, 1987, 1989) following diurnal courses of  $\pi$  and  $P$  and hence water potential ( $\psi = P - \pi$ ) in bladder and mesophyll cells. There was a clear water potential gradient between these two tissues at midday, constituting a driving force for transport of water from the bladders to the

mesophyll cells, demonstrating the protective function of the water storage tissue at critical times of stress (see Lüttge, 2002a). Water movement and diel cycles of internal water distribution between water-storage hydrenchyma and photosynthetic chlorenchyma has also been demonstrated for *A. deserti*, *Ferocactus acanthodes* (Tissue *et al.*, 1991) and *Opuntia ficus-indica* (Goldstein *et al.*, 1991).

Water storing hydrenchymas are not green, and generally are considered not to participate in metabolic functions of CAM. Bladder cells of *M. crystallinum* have no function in this respect (Winter and Lüttge, 1976; Winter *et al.*, 1981). Hydrenchyma of *Crassula falcata* might contribute to nocturnal CO<sub>2</sub> fixation (Springer and Outlaw, 1988).

CAM also occurs in some resurrection plants that are desiccation-tolerant and can shift between biosis and anabiosis as they dry out and are rewatered, respectively. The resurrection plants *Haberla rhodopensis* and *Ramonda serbica* (Gesneriaceae) perform various transitions between C<sub>3</sub> photosynthesis, CAM cycling and CAM idling as they dry out. CAM idling appears to be important during the first days after rewatering (Markovska *et al.*, 1997; Markovska 1999).

#### *Light (hv)*

Light has two important functions in CAM. First, it acts as the energy source of photosynthesis and second, it affects expression and performance of CAM via signalling systems.

*Light and photosynthesis* Intensity of photosynthetically active radiation (PAR, or photosynthetic photon flux density, PPFD), during the day (phase III) determines the rate of organic acid mobilization from the vacuole (Kluge, 1968; Barrow and Cockburn, 1982; Thomas *et al.*, 1987). The rate-limiting step in this process may be the directly light-dependent assimilation of CO<sub>2</sub> via Rubisco in the chloroplasts, malate decarboxylation in the cytosol, or malate efflux from the vacuole (Lüttge, 2002b). The high  $p^i_{CO_2}$  built up in phase III is evidently important in the regulation of the process, where a central role is played by carbonic anhydrase mediating the pH-dependent HCO<sub>3</sub><sup>-</sup>/CO<sub>2</sub> equilibria in the cytosol and chloroplast stroma, and the supply of the proper substrate CO<sub>2</sub> to Rubisco. To date, this enzyme has been unduly neglected in CAM research (Tsuzuki *et al.*, 1982; Holtum *et al.*, 1984; Raven and Spicer, 1996; Lüttge, 2002b). PPFD during the light period determines the degree of nocturnal organic acid accumulation during the subsequent dark period, i.e. the amplitude of the day/night oscillations of CAM, because light-driven photosynthesis and gluconeogenesis fill the carbohydrate stores required for PEP synthesis via glycolysis during the dark period as a precursor for phase I CO<sub>2</sub> fixation by PEPC (Nobel and Hartsock, 1983).

The energy requirement for carbon flow of the CAM cycle is higher than in C<sub>3</sub> photosynthesis. Estimates of stoichiometries of ATP : NADPH : CO<sub>2</sub> are 3 : 2 : 1 for C<sub>3</sub> photosynthesis and 4.8 : 3.2 : 1 for CAM with malate oscillations, and up to 5.9 : 3.9 : 1 for CAM with malate plus citrate oscillations (Winter and Smith, 1996b). It may be asked then, if CAM can get under energy limitation. This

may be the case for epiphytic CAM plants in very moist cloud forests during the rainy season (Pierce *et al.*, 2002). However, this may be the exception, as generally PPFD in CAM plant habitats is not limiting (Lüttge, 2002b).

Conversely, there may be surplus irradiance and over-energization of the photosynthetic apparatus in CAM. This is normally observed in C<sub>3</sub> plants at sun-exposed sites and elicits the various mechanisms of non-photochemical energy dissipation and photo-protective, as well as photo-destructive, photoinhibition. With the strong interactions of some input factors (Fig. 1) these processes are not governed by irradiance alone. Although in *Kalanchoë daigremontiana* water stress *per se* did not affect primary photochemical activity, e.g. potential quantum yield of photosystem II when leaves were darkened overnight, it increased susceptibility to photoinhibitory light stress during the day (Lu *et al.*, 2003). It has often been argued that the high  $p^i_{CO_2}$  built up in phase III of CAM protects plants from over-energization during the time of the day when the highest irradiance prevails due to substrate-saturated photochemical work using most of the excitation energy of photosynthesis (Gil, 1986). However, it has already been noted, whilst discussing the factor CO<sub>2</sub> above, that this is not the case. For protection, CAM plants possess the entire complement of energy dissipation methods known also from C<sub>3</sub> plants, namely photorespiration, radiative energy dissipation via zeaxanthin and the futile xanthophyll cycle of epoxidation and deepoxidation, D1-protein turnover, etc. (reviewed by Lüttge, 2000, 2002b; and more recently by Lu *et al.*, 2003).

*Light and signalling* High light intensities may elicit CAM expression in C<sub>3</sub>/CAM intermediate species, such as *Guzmania monostachia* (Maxwell *et al.*, 1994, 1995, 1999; Maxwell, 2002) and *Clusia minor* (where it is under the control of an UV-A/blue light receptor) (Grams and Thiel, 2002).

A signalling function of light related to CAM is obvious in the photoperiod, i.e. long-day dependent induction of CAM in the facultative CAM plant *Kalanchoë blossfeldiana* ‘Tom Thumb’. Phytochrome, the red-light receptor involved in photoperiodisms, elicits CAM expression (Brulfert *et al.*, 1973, 1975). Long-days also enhance CAM expression in *M. crystallinum* (Cheng and Edwards, 1991; Guralnick *et al.*, 2001a), where CAM induction is known to be mostly related to salinity and drought stress, and phytochrome is involved (Cockburn *et al.*, 1996). In *K. blossfeldiana* ‘Tom Thumb’ long-day regimes induce both flowering and a shift from C<sub>3</sub> photosynthesis to CAM (Brulfert *et al.*, 1973). Both flowering and CAM in plants are controlled by the ‘biological clock’, where blue light and red light are basically important environmental input factors, and cryptochromes (blue light receptors) and phytochromes are essential elements of input pathways (Lüttge, 2003a, b). To date, it is not clear where the output pathways that produce the overt phenomena of flowering and CAM branch. Other species of *Kalanchoë*, such as *K. daigremontiana* and *K. tubiflora*, although also long-day plants for the induction of flowering, are obligate CAM plants.

In C<sub>3</sub>/CAM intermediate species, light responses of stomata also change dramatically when CAM is induced. In *Portulacaria afra*, blue-light and red-light responses of stomata in the C<sub>3</sub>-state are lost in the CAM-state. Signals such as  $p^i\text{CO}_2$ , high water-vapour pressure differences between leaves and the atmosphere, high temperature and low water potential are excluded as being inhibitory signals for stomatal opening in response to blue and red light during CAM. The inhibition is also observed in isolated epidermal peels and different signalling must be involved (Lee and Assmann, 1992). The xanthophyll zeaxanthin is probably involved in the signal transduction chain from light to stomatal opening (Zhu *et al.*, 1998). In *M. crystallinum* after the C<sub>3</sub>–CAM transition, the opening response of guard cells to blue and white light is lost, together with light-dependent xanthophyll formation (Tallman *et al.*, 1997).

#### Temperature (T)

The major interactions of the temperature factor determining CAM performance are with individual enzymes, membranes, respiratory activity and stomatal movement. Temperature often may not be decisive *per se* but acts by modulating the impact of other factors (Kluge and Ting, 1978). Nobel (1996) notes that under conditions of cultivation, temperature is not a major factor affecting the productivity of CAM plants.

The contention that for optimal performance of CAM plants need relatively low night temperatures and high day temperatures goes back to *in vitro* studies by Brandon (1967) of temperature optima of key enzymes in the metabolic pathway, where the enzymes of nocturnal malate synthesis, PEPC and malate dehydrogenase reach their optimum at 35 °C and the decarboxylating enzymes, e.g. malic enzyme, at above 53 °C. *In vitro* PEPC enzymology shows that the active phosphorylated form of the enzyme is stabilized at low temperatures (3 °C or less) while higher temperature promotes dephosphorylation (Carter *et al.*, 1995), so that inhibition of the enzyme by allosteric effectors is also lower at low and higher at high temperatures (Buchanan-Bollig and Kluge, 1981; Carter *et al.*, 1995). Thus, from overall performance of the counteracting enzymes of carboxylation and decarboxylation, where lower temperatures favour the former and higher temperatures the latter (Buchanan-Bollig *et al.*, 1984), it was concluded that rather cool night temperatures somewhat below 20 °C would be most favourable for dark fixation in CAM. However, due to the complexity of temperature interactions and the frequent temperature acclimation, considering temperature optima of enzymes *in vitro* is a simplification. Comparing three growth temperature regimes, Israel and Nobel (1995) found that PEPC and Rubisco had maximal activities at 45/35 °C day/night while total daily CO<sub>2</sub> uptake was greater at 30/20 °C and 15/5 °C.

Another target of temperature is the cell membranes, where temperature directly affects fluidity, and hence permeability, which is very important for organic acid compartmentation (Friemert *et al.*, 1988). An inverse relation of diurnal heat tolerance of CAM plants to tissue acid levels is probably related to this, when higher

temperatures increase tonoplast permeability and acid efflux from the vacuole and the acid load may exert detrimental effects in the cytosol (Kappen and Lösch, 1984; Lehrum *et al.*, 1987). The tonoplast of CAM plants also shows very pronounced acclimation to growth temperatures. In a process of homeoviscous adaptation in *K. daigremontiana*, the membrane order or rigidity is much enhanced at growth temperatures of 34/25 °C day/night compared with that of 25/17 °C. This reduces malic acid permeability of the tonoplast and allows controlled malate accumulation/remobilization during the CAM cycle at elevated temperatures. Homeoviscous adaptation is due both to changed lipid composition and to lipid/protein interactions (Kluge *et al.*, 1991b; Kliemchen *et al.*, 1993; Behzadipour *et al.*, 1998).

Compartmentation of malic acid modulated by temperature effects on the tonoplast is also relevant for interactions with respiration and associated acclimation (Medina and Osmond, 1981). Labelling studies with stable carbon isotopes have shown that the flux of malate through mitochondria was approx. 100 % at the beginning, 60 % in the middle and 70–100 % at the end of the dark period. This is related to the CO<sub>2</sub> fixation rate, which is highest in the middle of the night when a higher proportion of the malate formed may first pass the mitochondria, and to the electrochemical energy gradient at the tonoplast against which malic acid is accumulated, which is highest at the end of the night (Kalt *et al.*, 1990). In these phytotron experiments, temperature was kept constant throughout the whole night. However, in the field, where temperature gradually decreases during the night towards the early morning, such interactions will be more important.

Temperature effects on air humidity may largely determine relationships between temperature and stomatal opening. Stomata of the CAM plant *K. pinnata* are highly sensitive to air humidity (Medina, 1982). Maximum rates of dark CO<sub>2</sub> fixation were similar at all temperatures between 12 and 25 °C within a given range of leaf/air water vapour pressure differences. However, the onset of nocturnal net dark CO<sub>2</sub> fixation and the time to reach a peak rate were delayed as temperature increased, and hence total CO<sub>2</sub> uptake and malate accumulation were reduced with increasing temperature during the dark period.

In summary, temperature relations suggest that diurnal temperature changes with lower night-time and higher daytime temperatures are favourable for CAM. In the C<sub>3</sub>/CAM intermediate *Clusia minor*, C<sub>3</sub> to CAM shifts are strongly enhanced when day/night temperature differences are increased and are less sensitive to absolute temperatures (Haag-Kerwer *et al.*, 1992). Conversely, there is also a large amount of evidence that CAM can be well expressed under constant temperatures (e.g. Lütte and Beck, 1992). Frequently in the tropics, day and night temperatures are not very different and CAM plants grow well and show lush occupation of such habitats (Kluge and Ting, 1978; Plant, Cell and Environment, 1986).

#### Nutrients

There is a lot of information on nutrient relationships of epiphytic CAM plants, because mineral nutrition in the

epiphytic habitat has been studied extensively. However, problems of mineral nutrient acquisition and particular solutions such as the formation of tanks and other phytotelmata, epiphytic root systems and mycorrhiza and myrmecophytism pertain to epiphytes in general and are not CAM-specific, although CAM plants obviously also make use of these adaptations (Benzing, 1983, 1990; Lüttge, 1989a).

Nutrient levels and element responses have also been studied in CAM desert succulents (Nobel, 1983; Nobel and Berry, 1985). In the chlorenchyma of cultivated cacti and agaves, levels of Ca, Mg and Mn tended to be higher than in most other agronomic plants. The rather high levels of Ca in these plants are noteworthy, and are also observed in many other CAM taxa such as *Aloe* and Crassulaceae (*Crassula*, *Kalanchoë*; Karmarkar and Joshi, 1969; Phillips and Jennings, 1976; Rössner and Popp, 1986; Meyer and Popp, 1997) as well as *Clusia* (Ball *et al.*, 1991a; Oliavares and Aguiar, 2002), and thus these CAM plants are calcitrophic species. Together with K<sup>+</sup>, Na<sup>+</sup> and Mg<sup>2+</sup>, Ca<sup>2+</sup> serves as a counter-ion for a background pool of carboxylates (of several tens of millimolar in some cases), which does not oscillate diurnally and is thought to help in osmotic stabilization (Phillips, 1980; Smith *et al.*, 1996). With a plethora of ionic dissociation equilibria involved, it is not easy to measure and calculate to what extent calcium is free or bound and sometimes contrasting conclusions are reached (Schomburg, 1994; Meyer and Popp, 1997; Behzadipour, 1999). This is important, because Ca<sup>2+</sup> can bind to negatively charged groups of proteins and lipids, and hence decrease membrane fluidity/permeability (Schomburg, 1994; Behzadipour, 1999). Although this might change diurnally during the CAM cycle in relation to changing levels of Ca<sup>2+</sup> binding organic acids, and might be involved in the regulation of switches between net acid accumulation and remobilization (Kluge and Schomburg, 1996), so far, there is no unequivocal evidence for such a mechanism (Schomburg, 1994; Meyer and Popp, 1997; Behzadipour, 1999).

The importance of N has been evaluated on theoretical grounds because it may be predicted that CAM plants might need less N than C<sub>3</sub> plants, and thus have a higher nitrogen use efficiency (NUE) (Griffiths, 1989; Raven and Spicer, 1996). In C<sub>3</sub> plants, Rubisco may account for 50 % or more of the total soluble leaf protein (Björkman *et al.*, 1976; Ku *et al.*, 1979), but CAM plants would need less Rubisco due to their CO<sub>2</sub>-concentrating mechanism, and hence bind less N in the Rubisco protein. Enzyme analyses in *K. pinnata* by Winter *et al.* (1982b) support this expectation. Since it is known that in *Kalanchoë* species CAM expression is related to leaf age and increases as leaves mature, they analysed activities and amounts of PEPC and Rubisco in leaves of increasing age and CAM expression. Activity and amount of Rubisco decreased while PEPC increased. In leaves of plants that were supplied with nitrate, the amount of enzyme protein related to total soluble protein in young and mature leaves was, respectively, 30 and 17 % for Rubisco and 1 and 10 % for PEPC. Nitrate and phosphate deficiency have positive effects on CAM expression in *M. crystallinum* (Paul and Cockburn, 1990) and N deficiency has positive

effects on CAM performance in *K. blossfeldiana* (Ota, 1988a). Santos and Salema (1991, 1992) studied mineral nutrition of the facultative CAM species *K. lateritia*. CAM was best expressed at intermediate N supply.

Conversely, it could be argued that the high levels of  $p^i\text{CO}_2$  oversaturating Rubisco in phase III of CAM could possibly make it beneficial for CAM plants to have more of the enzyme. Widmann *et al.* (1990) found that in *K. daigremontiana* and *K. tubiflora* NUE was less than expected for CAM plants. The ability of succulent C<sub>3</sub> and CAM species to use N was highly species-specific and varied with age and environmental conditions. CAM plants were no better adapted to N-deficient habitats than C<sub>3</sub> plants, which could use limiting N more efficiently by increasing transpiration and exploiting the soil more effectively (Widmann *et al.*, 1993). In the aquatic CAM plant *Littorella uniflora*, CAM did not increase NUE (Baatrup-Pedersen and Madsen, 1999).

Contrasting observations are also related to NH<sub>4</sub><sup>+</sup> vs. NO<sub>3</sub><sup>-</sup> preference of CAM plants. Ota (1988b) and Ota *et al.* (1988) found that in *K. blossfeldiana* CAM was more pronounced under NO<sub>3</sub><sup>-</sup> than under NH<sub>4</sub><sup>+</sup> nutrition. In both cases, drought stress increased CAM performance but the discrepancy between NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> treatments increased. It was suggested that NH<sub>4</sub><sup>+</sup> depresses PEPC activity and CAM (Ota and Yamamoto, 1991). Conversely, Fernandes *et al.* (2002) found that when tanks of the terrestrial CAM bromeliad *Neoregelia cruenta* were supplied with 5 mM NH<sub>4</sub>NO<sub>3</sub> there was a preference for NH<sub>4</sub><sup>+</sup> uptake over NO<sub>3</sub><sup>-</sup> uptake of more than 11-fold within 24 h. In two other bromeliads, NO<sub>3</sub><sup>-</sup> was found to be a poor N source but was effective together with NH<sub>4</sub><sup>+</sup> (Nievola *et al.*, 2001). It could be argued that the use of reduced N, including organic N, is a specific adaptation of tank-forming bromeliads where putrefying litter and dead animals produce reduced N and low molecular organic N compounds which may be particularly important in the epiphytic habitat (Endres and Mercier, 2001, 2003). On the other hand, *Clusia* species fed via their roots also use reduced N (Wanek *et al.*, 2002a) and Arndt *et al.* (2002) observed that although potentially all N sources supplied to the roots of *C. minor* could be used, there was a strong preference for NH<sub>4</sub><sup>+</sup> over NO<sub>3</sub><sup>-</sup> and glycine.

Ecophysiological it is important to consider the N factor in relation to the action of other factors, especially light and water. Generally, chlorophyll contents, rates of photosynthesis and quantum yield decline under N limitation and this also holds true for some factor combinations in CAM plants, e.g. the bromeliads *Bromelia humilis* (Fetene *et al.*, 1990) and *N. cruenta* (Fernandes *et al.*, 2002), and *K. pinnata* (Lüttge *et al.*, 1991a). With the latter, a multifactor study was performed where plants were grown at high and low irradiance, with and without N- and H<sub>2</sub>O-deficiency and analysed with and without transfer from low to high and from high to low irradiance (Lüttge *et al.* 1991a, b). The plants showed highly varying NUE under these different conditions. Sufficient N supply overall had positive effects. It also allowed flexible responses to irradiance. For example, when well-watered and N-supplied shade-grown plants were transferred to high light conditions, total

day/night net CO<sub>2</sub> uptake was much increased, particularly due to phase IV photosynthesis. Shade plants when well supplied with N were not photoinhibited in high light. Similar observations were made by Fernandes *et al.* (2002) with *N. cruenta* where high light and high N leaves behaved similarly to shade plants. In *B. humulis*, NUE (expressed as mol CO<sub>2</sub> taken up per mole leaf N) was reduced by more than 50 % by N deficiency in both high light and low light grown plants (Fetene *et al.*, 1990). Nitrate nutrition at high light also promoted CAM performance in *C. minor* (Franco *et al.*, 1991).

Overall, there is no clear evidence supporting particularly high NUE in CAM plants. Multifactorial effects and responses in relation to N nutrition of CAM plants obviously show a larger variability than any differences between C<sub>3</sub> and CAM plants.

#### Salinity

One of the major effects of salinity is osmotic stress, and hence there are intimate relationships to drought stress or 'water stress'. Therefore, considering CAM as a major photosynthetic accommodation to water stress, CAM might be expected to be a prominent trait among halophytes. Moreover, halophytes are often succulent as they sequester NaCl in large central vacuoles, which is called 'salt succulence' (Ellenberg, 1981). It might be speculated that this is a good preadaptation for CAM, inasmuch as it is argued that the development of succulent leaf anatomy with large uniform spherical cells, typical of many CAM plants (e.g. *Kalanchoës*), is a first step towards the evolution of CAM (Guralnick and Jackson, 2001; Guralnick *et al.*, 2001b).

However, observations do not support this expectation as, in general, halophytes are not CAM plants and CAM plants are not halophytes. There are some isolated reports that CAM plants may cope well with mild NaCl stress, e.g. individuals of coastal populations of the cactus *Opuntia humifusa* (Silverman *et al.*, 1988) and some Macaronesian Sempervivioideae (Lösch and Kappen, 1981; Lösch 1990), but generally CAM plants, including desert succulents, are highly salt sensitive (Nobel, 1983; Nobel and Berry, 1985). CAM plants inhabiting highly saline ecosystems, such as coastal and inland salinas, are effectively functional salt excluders at the root level, such as some cacti (Nobel *et al.*, 1984) and, mainly, stress avoiders displaying either morphological root system dynamics in response to periodic stress or complete escape from the saline substratum by retreat to epiphytic niches.

The single conspicuous exception to the above is the annual facultative halophyte and facultative CAM species *Mesembryanthemum crystallinum* (Lüttge, 1993, 2002a; Adams *et al.*, 1998; Cushman and Bohnert, 2002). This plant can grow well in the absence of NaCl but has its growth optimum at several hundred mM NaCl in the medium and can complete its life cycle at 500 mM NaCl (Winter, 1973; Lüttge, 2002a). It is a species with inducible CAM. It exclusively performs C<sub>3</sub> photosynthesis during the early stages of its growth when kept at low salinity. Although it has an inherent developmental programme that

gradually shifts its metabolism to CAM, even if continuously kept at low salinity, high salinity rapidly induces CAM in young plants and strongly enhances the age-dependent CAM (Adams *et al.*, 1998; Lüttge, 2002a). Many responses to salinity and CAM induction have been described. At the membrane level, the proton pumping V-ATPase of the tonoplast is enhanced while the proton pumping V-pyrophosphatase is down-regulated. Changes to membrane fluidity suggest molecular modifications of the tonoplast. At the cellular level, there is a change of photosynthetic metabolism based on inductive changes of gene expression at the molecular level with a regulatory network involving phytohormones and secondary messengers and where transcriptional and post-translational regulation also interact. This has been covered frequently in other reviews (Lüttge, 1993; Cushman and Bohnert, 1997, 1999, 2002; Cushman, 2001) so the keynotes given above will suffice here. The inductive responses of gene expression in *M. crystallinum* also offered the opportunity to identify CAM-specific enzymes (Meyer *et al.*, 1990). Although the enzymatic machinery required for CAM is not basically different from the complement of housekeeping enzymes, the identification of new isoforms of enzymes after the induction of CAM in *M. crystallinum* helps in the identification of particularly important key enzymes of CAM.

#### RECEIVERS: PLANT TYPES

The receivers of environmental inputs are the various types of plants. Basically, we distinguish genotypes and phenotypes. The genotype comprises the complete genetic information of the plant, and thus represents a given constitution. As such it is not affected by environmental input. Due to a genetic developmental programme ontogenetically it generates a certain phenotype, which is always the direct receiver of environmental input. Responses of gene expression to environmental input are due to feedback from the phenotype, e.g. in *M. crystallinum* where there is a developmental programme with expression of CAM as the plants age but where environmental stresses, such as salinity and drought, accelerate CAM expression in time and enhance the degree of CAM expression.

#### Genotypes

To date, there is insufficient access to CAM genotypes. With the planned sequencing of *M. crystallinum*'s genome (Meyer *et al.*, 1990; Adams *et al.*, 1998), it may be possible, at some time, to handle complete genomic information of a C<sub>3</sub>/CAM intermediate species. Moreover, there are mutants of *M. crystallinum*, one of which is CAM deficient (J. Cushman, pers. comm.), and this may advance our understanding of the extent to which there is genomic anchorage of the CAM syndrome.

Hence, at the genetic level, we remain restricted to indirect information, as given by molecular analyses of isoenzymes essential for CAM. Genes may differ for isoenzymes within a given plant species, for example PEPC expressed in various plant organs and serving

housekeeping functions and CAM-type dark fixation of CO<sub>2</sub>, respectively (Gehrig *et al.*, 1998). Different genes may encode isoenzymes of C<sub>3</sub> and CAM plants, or different isoenzymes before and after induction of CAM in C<sub>3</sub>/CAM intermediate species (Brulfert *et al.*, 1982, 1985; Brulfert and Queiroz, 1982; Groenhof *et al.*, 1990; Slocombe *et al.*, 1993; Gehrig *et al.*, 1995). Following the evolutionary genetic trend in conservation and diversification, respectively, of key enzymes, e.g. PEPC, is another important approach.

Some information is also being gradually gathered by molecular studies of phylogenetic relationships among CAM taxa. Because of the relationships between genotypes and phenotypes, it is important in ecology to obtain comparative genetic information so that molecular analyses of populations become increasingly essential in eco-physiology. For CAM, research, to date, has been mostly restricted to the family Bromeliaceae and the genera *Clusia* and *Kalanchoë*. The research is based on the detection of DNA polymorphisms by DNA amplification from arbitrary short primers (RAPD-PCR fingerprinting) and, more recently, on the study of internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA.

*Clusia* is a large genus of neotropical species of woody plants and trees. The number of species is estimated to be approx. 300 (Pipoly *et al.*, 1998). The family Clusiaceae and the subfamily Clusioideae with the genus *Clusia* have a minimum phylogenetic age of  $90 \times 10^6$  years (Gustafsson *et al.*, 2002). ITS sequencing of nuclear ribosomal DNA has been used by two independent groups to determine the molecular phylogeny of *Clusia*. Vaasen *et al.* (2002) studied 17 species and Gehrig *et al.* (2003) studied 31 species, with 12 species being common to both studies. *Clusia* is considered as being a monophyletic genus. The two studies both arrived at the conclusion that in the genus CAM developed independently more than once, i.e. the idea that CAM may have evolved only once from a C<sub>3</sub> ancestor of *Clusia* is ruled out. It is now considered incorrect that all *Clusia* species might have at least a small and weak capacity for CAM, as proposed by Grams *et al.* (1998). *Clusia multiflora*, which is currently taken as the ‘model C<sub>3</sub> species’ of the genus, is not at the bottom of the molecular phylogenetic trees. In other respects, observations and conclusions of the two research groups are variable. Vaasen *et al.* (2002) did not find agreement between their molecular data and phylogenetic trees based on morphological studies of flowers, fruits and vegetative parts. However, Gehrig *et al.* (2003) found close correlations between their cladograms of Panamanian *Clusia* species and three morphologically defined groups of Central American *Clusias*. Considering the large variety of CAM physiotypes (with very weak to strong CAM) which *Clusia* genotypes can generate and the scarcity of *bona fide* obligate C<sub>3</sub> species, one group thinks that CAM is dominant in the genus (Lüttge, 1996, 2000; Vaasen *et al.*, 2002) while the other group appears to consider that ‘some species of *Clusia* exhibit CAM’ (Gehrig *et al.*, 2003).

The Bromeliaceae comprise approx. 2800 species (Luther and Sieff, 1998). Martin (1994) has identified the photosynthetic pathway of 249 species, of which 69 % show

CAM capacity with varying degrees of CAM expression. The family itself is considered monophyletic. Among the three subfamilies, the Bromelioideae are probably monophyletic and the Tillandsioideae possibly so, while the Pitcarnioideae are definitely polyphyletic (Crayn *et al.*, 2000). CAM has evolved polyphyletically several times within each subfamily (Smith, 1989; Crayn *et al.*, 2000). Thus, again it is difficult, if not impossible, to trace a specific CAM genome.

The only obvious case where CAM is related to phylogenetic sections is in the genus *Kalanchoë*. According to the taxonomy of Boiteau and Allorge-Boiteau (1995) there are three sections, marking an evolutionary trend from *Kitchingia* to *Bryophyllum* to *Eukalanchoe*. Comparative DNA polymorphism and ITS sequencing of nuclear ribosomal DNA confirm these relationships based on morphological characteristics, and the degree of CAM expression follows the same evolutionary trend. First, facultative, drought-inducible but, once induced, extremely flexible CAM is found in all groups of thin-leaved, not very succulent species in Boiteau’s section *Kitchingia* and in the first two groups of the section *Bryophyllum*. Second, obligate, but flexible, CAM (especially with respect to making use of daytime phase IV CO<sub>2</sub> uptake) is found in the remaining groups of the section *Bryophyllum*. Third, obligate, uniform CAM is found in the section *Eukalanchoe* (Gehrig *et al.*, 1997, 2000). Thus, the coincidence between the degree of CAM expression and the infrageneric phylogenetic position of the species suggests that the diversity of CAM pattern in the genus is not due solely to phenotypic flexibility but is largely determined genetically. Hence, in this genus CAM may indeed have evolved monophyletically from an ancestor with dominating CO<sub>2</sub> acquisition by the C<sub>3</sub> pathway and only weak nocturnal CO<sub>2</sub> fixation gradually advancing to the development of full CAM. The genus is also a good example of how separation of populations and segregation may have driven speciation and evolution. The diversification centre of *Kalanchoë* is the large island of Madagascar. The centre of adaptive radiation is thought to be located in the humid regions on the eastern side (Boiteau and Allorge-Boiteau, 1995) where CAM does not appear to be of selectional advantage and from where plants have spread into the arid regions in the dry south and from there to arid sites in eastern continental Africa (Gehrig *et al.*, 2001). Currently, this is still reflected by the distribution of species of the three sections with weak, medium and strong CAM at the community level in the different ecosystems of the island.

#### Phenotypes

For phenotypes, morphotypes and physiotypes can be distinguished. An invaluable checklist of CAM plants documented over the past 25 years with information on morphotypes and physiotypes has been produced by Sayed (2001; see also Smith and Winter, 1996). The morphotypes are structural life forms, as delineated by comparative morphology and anatomy. The physiotypes are physiological life forms as delineated by comparative physiology, biochemistry, biophysics and molecular biology.

TABLE 4. Morphotypes of CAM plants (examples of families, genera and species)

Physiognomy	Remarks	Examples
Leaf succulents	Large uniform cells with large central vacuole Water storage tissue peripheral, epidermal hypodermal	Crassulaceae <i>Mesembryanthemum crystallinum</i> <i>Agave</i> , <i>Aloë</i> , Bromeliaceae, <i>Peperomia</i>
Stem succulents	Reduction of surface due to leaflessness, central water storage tissue	Didieraceae, Cactaceae, Euphorbiaceae, Asteraceae, Apocynaceae, Asclepiadaceae
Epiphytes		Orchidaceae, Bromeliaceae, <i>Peperomia</i> , some Cactaceae
Climbers and stranglers		Some Cactaceae, <i>Clusia</i>
Rosettes	Terrestrial rosettes Stem supported rosettes Tank-forming rosettes Submerged freshwater rosettes	<i>Agave</i> , <i>Aloë</i> , Bromeliaceae, <i>Sempervivum</i> <i>Yucca</i> Bromeliaceae Isoetaceae: <i>Isoetes</i> , <i>Stylites</i> ; <i>Sagittaria</i> , <i>Crassula</i> , <i>Littorella</i>
'Trees'	Large stem succulent dicotyledons Monocotyledonous-type secondary thickening True dicotyledonous trees	Didieraceae, Cactaceae, Euphorbiaceae <i>Yucca</i> <i>Clusia</i>

**Morphotypes.** In physical appearance, there is no typical morphotype of CAM plants. CAM is expressed in a large range of morphologically very different life forms. There is only one morphological/anatomical feature that is common to all CAM plants, namely more or less strongly pronounced succulence. This is often, but not only, due to the formation of large water storage tissues, because nocturnal organic acid accumulation and the associated osmotic adjustments in the photosynthetically active tissue always require large cells with the central vacuole occupying a high proportion (up to 98 %) of the total cell volume (Steudle *et al.*, 1980; Lüttge and Smith, 1984). Such succulence has even been considered as a prerequisite for the evolution of the biochemical CAM cycle (Guralnick and Jackson, 2001; Guralnick *et al.*, 2001b). CAM expression and leaf succulence are often closely correlated (Teeri *et al.*, 1981; Winter *et al.*, 1983; Kluge *et al.*, 2001).

Table 4 gives an overview of CAM plant morphotypes. The data in the table do not aim to be systematic or complete; several of the patterns distinguished overlap, e.g. rosettes are also leaf-succulents, etc. However, it may help to give a general overview (see also table in Sayed, 2001). Simple CAM leaf-succulents, such as *Kalanchoë* species, are uniformly composed of large spherical cells. Other CAM leaf-succulents have special non-green water storage tissues that can be peripheral, hypodermal or central in the mesophyll. Stem-succulents are characteristic life forms of arid sites and deserts and have central water storage tissues. Many epiphytes are CAM plants, most being either bromeliads or orchids although some belong to other taxa. Epiphytic cacti are normally spineless. Some cacti are liana-like climbers. All Cactaceae perform CAM, but among the three subfamilies, Pereskioideae, Opuntioideae and Ceroideae, the Pereskioideae grow leaves seasonally and leaves may also occur in the Opuntioideae, and those leaves perform C<sub>3</sub> photosynthesis while the succulent green cladodes and stems perform CAM (Nobel and Hartsock, 1987). Many CAM plants are rosettes, some with special features like the water-storing phytotelmata of tank-forming bromeliads. Leaves of submerged, freshwater rosette plants perform CAM, but in amphibious plants CAM does not

occur when the leaves are exposed to the air (Keeley, 1996; Robe and Griffiths, 2000). Thus, CAM is distributed among all kinds of morphotypes, providing various potential adaptive advantages, but there is no single one that appears particularly well suited for CAM. An enigma is posed by the last line of Table 4. Why are there almost no CAM trees? Among stem-succulent dicotyledons there are species that reach a spectacular size, for example columnar cacti, candelabrum euphorbias and some Didieraceae, which have been called 'tree-succulents' (Ellenberg, 1981). Moreover, Vareschi (1980) has referred to cactus forests. However, these are not real trees with secondary thickening growth, and the sensation of being in a forest with a closed canopy when walking in such habitats does not so much originate from the columnar cacti but rather from the acacias and other woody plants scattered among them. Moreover, there is the CAM-performing Joshua tree of the genus *Yucca* with a special monocotyledonous type of secondary thickening. However, the only true dicotyledonous trees performing CAM are species of *Clusia*. The question why there are not more dicotyledonous trees with CAM cannot, at present, be answered. The great success of CAM *Clusiias*, with an extraordinarily large ecological amplitude covering coastal sites of rocks and dunes, savannas, rock outcrops, gallery forests, rainforests and cloud forests (Lüttge, 1996), seems to rule out that tree structure and CAM function are not compatible. Is it the high plasticity, flexibility and speciation rate within the genus *Clusia* that led to a first evolution of CAM among trees?

Different life stages of CAM plants may involve morphotypic differences. Often it is simply plant size that affects ecophysiological performance, particularly with respect to water relations (Schmidt *et al.*, 2001; Zotz *et al.*, 2001; Hietz and Wanek, 2003). In stem-succulent CAM plants, the larger surface/volume ratio of small plants compared with that of larger ones leads to a higher relative water loss in the former compared with that of the latter and makes survival under extended dry conditions more difficult (Jordan and Nobel, 1979, 1982; Lerdan *et al.*, 1992).

However, different life stages may produce more clearly different morphotypes. *Clusiias* are hemiepiphytic

stranglers. Seedlings may start growth terrestrially and develop as independent trees. Alternatively, they may start growth epiphytically and grow aerial roots, some of which gain soil contact while others act as holdfasts and eventually strangle the host, resulting in *Clusia* eventually becoming an independent tree as the host dies and rots away (Ball *et al.*, 1991a). However, the expression of CAM is strongly under environmental control and is not correlated with the life form of the seedling, epiphyte, strangler or tree. Thus, there is no ontogenetic programme for CAM expression (Ball *et al.*, 1991b; Wanek *et al.*, 2002b).

Among tank-forming, epiphytic CAM bromeliads, many species have a different morphotype in the juvenile stage. Juvenile plants form small rosettes of narrow leaves not overlapping at their bases and thus, do not form phytotelmata (Schmidt and Zotz, 2001). They therefore have particular problems of water and nutrient acquisition similar to the so-called atmospheric bromeliads which can attach themselves to any support, including fence wires and telephone cables, and are restricted to the absorption of water and nutrients, via epidermal scales or trichomes, from rain, aerosols and dust. Juvenile stem-succulents, e.g. cacti, are handicapped in not being able to store sufficient water compared with that of larger adult individuals, because the juvenile plants have larger surface area : volume or chlorenchyma : hydrenchyma ratios (Jordan and Nobel, 1979, 1982).

With respect to life cycles, most CAM plants are perennial. Many CAM plants are monocarpic or hapaxanthic, i.e. they die after flowering either in their second year as biennial plants, e.g. *Kalanchoës*, or after many years in the vegetative state once flowering has occurred, e.g. *Agaves*. Apart from *M. crystallinum* and *M. nodiflorum* (Winter and Troughton, 1978) and *Crassula siberiana*, an annual leaf-succulent growing in granitic outcrops in New South Wales, Australia (Brulfert *et al.*, 1991), there are no other reports of annual CAM plants.

**Physiotypes.** Photosynthetic physiotypes are the major physiotypes that can be distinguished for the metabolic cycle of CAM. ‘CAM cycling’, ‘full CAM’ and ‘CAM idling’ have already been mentioned above. Full CAM may show considerable plasticity due to the flexible expression of the various CAM phases, and this comprises CAM idling. It is also noteworthy that *bona fide* obligatory CAM plants may turn totally to C<sub>3</sub> photosynthesis under certain conditions, e.g. *K. daigremontiana* at 7 °C (Kluge, 1969). Even the extremely atmospheric CAM bromeliad *Tillandsia usneoides*, with its apparent strong constitution for endurance of stress, shows high plasticity and may perform C<sub>3</sub> photosynthesis to a considerable extent as conditions allow (Kluge *et al.*, 1973; Haslam *et al.*, 2002, 2003). However, all these flexible reactions of a given plant or species can still be regarded as comprising the ‘full CAM’ physiotype. Conversely, CAM cycling may be a separate physiotype, as it appears that some species only express CAM cycling and never advance to stronger CAM. This is also sometimes considered as a first step in the evolution of CAM.

In addition, there are the true intermediates (see also Winter and Smith, 1996b; Sayed, 2001). There are a fair number of C<sub>3</sub> photosynthesis/CAM intermediates that can switch between full C<sub>3</sub> photosynthesis and full CAM. Some of them have already been mentioned above where the impact of environmental factors eliciting such switches have been discussed, e.g. photoperiod in *K. blossfeldiana* ‘Tom Thumb’, salinity in *M. crystallinum*. *Guzmania monostachia* appears to be the only real C<sub>3</sub>/CAM intermediate species in the large CAM plant family Bromeliaceae (Maxwell *et al.*, 1994, 1995, 1999; Maxwell, 2002), although there is some evidence that *Nidularium innocentii* may also be C<sub>3</sub>/CAM (see Griffiths, 1989). The large genus *Clusia*, that has only one morphotype of entire and somewhat leathery succulent leaves, comprises three photosynthetic physiotypes, i.e. C<sub>3</sub>, C<sub>3</sub>/CAM and CAM (Lüttge, 1999). The list of species compiled by Sayed (2001) includes highlighting of CAM-inducible, i.e. C<sub>3</sub>/CAM, intermediate plants.

Sage (2002) has questioned whether CAM and C<sub>4</sub> photosynthesis are compatible. It appears, however, that there genuinely are some C<sub>4</sub>/CAM intermediate species, e.g. *Peperomia camptotricha* (Nishio and Ting, 1993), *Portulaca oleracea* (Koch and Kennedy, 1980, 1982; Mazen, 1996) and *Portulaca grandiflora* (Koch and Kennedy, 1980, 1982; Ku *et al.*, 1981; Kraybill and Martin, 1996; Guralnick and Jackson, 2001; Guralnick *et al.*, 2002). Only succulent C<sub>4</sub> dicotyledons are capable of diurnal fluctuations of organic acids, where dark-respiratory CO<sub>2</sub> is trapped in bundle sheaths by PEPC and the water storage tissue in the succulent leaves may also participate in the fixation of internally released CO<sub>2</sub> (Ku *et al.*, 1981). In *Portulaca*, this may be a form of CAM cycling in leaves with C<sub>4</sub> photosynthesis, while stems perform CAM idling (Guralnick *et al.*, 2002).

However, although C<sub>4</sub> photosynthesis and weak CAM occur in the same leaves, they are separated in space and do not occur in the same cells. This incompatibility of C<sub>4</sub> photosynthesis and CAM may be due to anatomical, biochemical and evolutionary incompatibilities. The separation of malate synthesis and decarboxylation in space in C<sub>4</sub> photosynthesis and in time in CAM, respectively, and the primary evolution of C<sub>4</sub> photosynthesis for scavenging photorespiratory CO<sub>2</sub> and of CAM for scavenging respiratory CO<sub>2</sub> (CAM cycling) may be the most important backgrounds of these incompatibilities. Although single cells may perform C<sub>4</sub> photosynthesis, there is intracellular compartmentation of carboxylation and decarboxylation, and these cells never perform CAM. Unlike C<sub>3</sub>-CAM coupling, there is never C<sub>4</sub>-CAM coupling and both pathways only occur side by side in C<sub>4</sub>/CAM intermediate species (Sage, 2002).

In summary, the CAM physiotypes CAM, CAM idling, C<sub>3</sub>/CAM and C<sub>4</sub>/CAM can be distinguished.

Roots have been largely neglected in CAM research. Of course, as CAM is a mode of photosynthesis, the focus has naturally been on the green tissues of CAM plants, but roots provide the essential support by the pedosphere and cannot be disregarded in the ecophysiology of CAM. Some information is available on green aerial roots of epiphytic

orchids that may perform CAM (Winter *et al.*, 1985; Goh and Kluge, 1989; Kluge *et al.*, 2001). A lot of information is available on epiphyte roots and their role in anchorage and nutrition including morphotypic distinctions of root systems of bromeliads, namely soil roots, tank roots and anchorage roots (Smith *et al.*, 1986; Smith, 1989). Cacti may sacrifice their fine roots during periods of severe drought stress, which they may overcome by many weeks of CAM idling with continuously closed stomata, and then rapidly regrow new roots when precipitation occurs and soil water becomes available again (Kausch, 1965; Lüttge *et al.*, 1989).

A systematic functional study of CAM root systems was performed on CAM desert succulents, i.e. cacti (mainly *Ferocactus acanthodes* and *Opuntia ficus-indica*) and agaves (mainly *Agave deserti*). The performance of these roots is conspicuous and may be considered as a pronounced CAM- or at least desert-succulent physiotype. The roots have rectifier properties and respond opportunistically to changing water potential ( $\psi$ ) gradients between soil and root tissue. When  $\psi_{\text{soil}} > \psi_{\text{root}}$  they maintain a high hydraulic conductivity,  $L_p$ , for water uptake. When the soil dries out and  $\psi_{\text{root}} > \psi_{\text{soil}}$ ,  $L_p$  is reduced so that water loss from the succulent stems and leaves via the roots is minimized. This is supported by the formation of soil/root air gaps as roots shrink in drying soil reducing root to soil contact, by formation of cortical lacunae in the roots and by peridermal dehydration. In addition to reduced radial  $L_p$ , axial hydraulic conductance is reduced by moderate reversible embolism and also by the formation of tyloses. In cases where radial  $L_p$  is increased during drying at root junctions and by local disruption of suberized cell layers at root surfaces, reduction of axial hydraulic conductance is essential. Reversibility of all these changes makes up for the rectifier nature of root performance, and is thus an important adaptive plasticity (Nobel and Sanderson, 1984; Nobel and Cui, 1992; North and Nobel, 1992, 1997, 1998; North *et al.*, 1993).

## OUTPUT

### *Large-scale impact of CAM at the community level*

When the input of environmental factors acts on the plant types as receivers, the output is the performance of plants at the community level in habitats and ecosystems. Considered for individual species, this is aut-ecology. But what is the syn-ecological impact of CAM? Are there globally zono-biomes *sensu* Heinrich Walter (Walter and Breckle, 1983), which are dominated by CAM plants? Are there at least habitats or ecosystems whose physiognomy is determined by CAM plants? Aut-ecologically, the biomass productivity of CAM is low compared with that of  $C_3$  and  $C_4$  plants (Table 5). Although cultivated CAM crops, mainly cacti and agaves, may reach productivity values comparable with those of  $C_3$  plants (Nobel, 1996), this occurs under technically advanced agricultural management and relies mainly on  $C_3$ -type phase IV  $\text{CO}_2$  acquisition. For naturally growing wild CAM plants, CAM is generally a strategy for stress survival and not for high productivity and dominance. Thus, global dominance of CAM cannot be observed in any zono-biome.

TABLE 5. Productivity of CAM plants compared with that of  $C_3$  and  $C_4$  plants

	Daily (g dry matter $\text{m}^{-2} \text{d}^{-1}$ )	Crops ( $\text{Mg ha}^{-1} \text{year}^{-1}$ )
CAM	1.5–1.8	40–50
$C_3$	50–200	35–45
$C_4$	400–500	50–90

Data after Black (1973; daily productivity) and Nobel (1996; crop productivity).

Although there are CAM species with respectable sizes among cacti, euphorbs, the Didieraceae and the dicotyledonous CAM trees of *Clusia*, it will be noted below when considering some of the major ecosystems with CAM plants that mainly their physiognomy is not dominated by CAM species.

Globally, there are some CAM plants in temperate zones, e.g. in northern deserts and prairies on the American continent (Cactaceae, Crassulaceae) and in mountains in the old world (Crassulaceae: *Sedum*, *Sempervivum*). The distribution of the CAM families Cactaceae and Crassulaceae on the North American continent was surveyed by Teeri *et al.* (1978). For both families, the distribution was highly negatively correlated with available soil moisture. CAM expression appeared to be of less overall importance to the Crassulaceae than to the Cactaceae. Both families reached as far north as 50° latitude (with individual cases still further north) but with decreasing abundance of native species as a percentage of total spermatophyte flora, i.e. maxima of 1.5–5.2 and 0.8 % between 30° and 40°N and 0.5 and 0.6 % between 40° and 50°N for the Cactaceae and the Crassulaceae, respectively. However, CAM plants are mainly concentrated in the subtropics and tropics, where they are found in contrasting habitats, i.e. at both very arid and very moist sites.

One notable example of a geographically large-scale analysis of CAM at the community level is for the genus *Kalanchoë* on the island of Madagascar (590 000 km<sup>2</sup>). The island has a climatic gradient from east to west of (1) perhumid-hot, (2) perhumid-mild, (3) humid-mild, (4) humid-cool and (5) humid-cold as the altitude increases from sea level to approx. 2800 m a.s.l., and then (6) subhumid-mild to cool, (7) subhumid-hot and (8) semiarid-hot going further west with decreasing altitude. A coastal strip in the farthest south-west of the island is (9) subarid-hot. The radiation centre of *Kalanchoë* is the humid east and species of the most basic section, *Kitchingia*, capable of weak CAM but normally performing  $C_3$  photosynthesis in the field, are restricted to the evergreen rain forest and evergreen montane forest of the first three climate types mentioned above. The other phylogenetic groups and physiotypes are further to the west, i.e. the sections *Bryophyllum* and *Eukalanchoë* with mostly obligate but flexible and strong obligate expression of CAM, respectively. The latter concentrate in the very arid south-west (Kluge *et al.*, 1991a). This is also the climatic zone of the island where succulent CAM plants of the Didieraceae (all

species of which perform CAM), the Euphorbiaceae and the Asclepidaceae are found (Winter, 1979; Kluge *et al.*, 1995). The progress of phylogeny of subgroups of the genus *Kalanchoë*, and linked to it evolution of the degree of CAM expression in the genus, is evident through studies at several relevant levels, namely morphological taxonomy backed up by molecular phylogeny (Gehrig *et al.*, 2001), phytogeography and ecophysiology (Kluge *et al.*, 1991a). This is one of the best examples of physiological syn-ecology so far known.

#### *Major ecosystems with CAM plants*

**Submerged aquatic sites.** Global distribution of submerged CAM water plants is described by Keeley (1996). For freshwater CAM plants the sites are seasonal pools, oligotrophic lakes, freshwater tidal creeks, irrigation channels and the like, all over the world, including high latitudes. As mentioned above, the earliest evolution of CAM might have occurred in submerged plants. However, the premium of CAM is on surviving abiotic stress and not dominance and high biomass production in these poor oligotrophic sites. For marine plants, the argument would be different if we accepted the limited overnight acid accumulation in the Phaeophyceae as CAM, in view of the large biomass production of kelp. Overall, although submerged plants may be extant reminders of the origin of CAM evolution, they do not constitute dominant CAM communities.

**Deserts.** The physiognomy of some deserts and, particularly, semi-deserts, on the American continent is characterized by large cacti and agaves, in Africa by stem succulent Euphorbiaceae and in Madagascar by the Didieraceae. Arid thorn-bush communities in Venezuela were even named ‘cactus forests’ (Vareschi, 1980). Hence, we may speak of typical CAM communities in these cases. Ellenberg (1981) has investigated the reasons for the phytogeographic distribution of stem-succulents in the arid zones of the globe. It should be noted that these large succulents do require seasonal precipitation, and that they are not truly drought resistant. They grow best with an average annual precipitation between 75 and 500 mm, i.e. they can withstand low precipitation, but precipitation must be regular. CAM succulents can overcome many weeks and even months by CAM idling but then they must fill the water stores of their hydrenchymas again. This is also seen in seedling establishment. With their larger surface : volume ratio, young seedlings and small plants can only survive short periods of drought. The severity and duration of dry periods in a seasonal desert climate determines whether or not seedlings can get established and survive. This has been shown by relating age classes of desert succulents to climate records (Jordan and Nobel, 1979, 1982). Thus, in very extreme deserts there are few, or no, CAM succulents. In the Negev desert in Israel, a few species of *Caralluma*, including *C. negevensis*, are the only stem-succulent CAM species, and they grow protected in the shade of rocks and benefit from the formation of dew after cold nights in these

niches (Lange *et al.*, 1975). In general, succulent CAM plants need regular rain. Cellular water potentials range between -0.1 and -1 MPa (Lüttge, 1985) and rarely get lower even under pronounced drought conditions, while non-CAM desert plants may attain values as low as -15 MPa (Richter, 1976). Thus, high variability of precipitation over many years may be the major reason for the lack of large stem-succulents in the deserts of Asia, Australia and the inner Sahara (Ellenberg, 1981).

In addition to water, another important factor in determining the phytogeographic distribution of CAM succulents is temperature. Stem-succulents have large temperature amplitudes but cannot withstand long periods of frost (Ellenberg, 1981). However, the range of temperatures tolerated by cacti and agaves is quite large. The highest soil surface temperature tolerated by some cacti is 74 °C on the bare soil where they grow, the highest temperature tolerance known for higher vascular plants (Nobel *et al.*, 1986). The photosynthetic electron transport apparatus of agaves and cacti also tolerates longer periods at higher temperatures than any other vascular plant (Chetti and Nobel, 1987). Acclimation to high temperatures occurs, and is associated with protein synthesis (Kee and Nobel, 1986; Nobel and de la Barrera, 2003). The lowest temperatures tolerated by cacti are -10 °C by *Opuntia ficus-indica* and *O. streptacantha* and -24 °C by *O. humifusa* (Goldstein and Nobel, 1994). This cold-tolerance is important for cacti that have reached northern latitudes in southern Canada and the eastern United States where there is seasonal cold-hardening and acclimation to sub-freezing temperatures in the winter (Nobel and Smith, 1983). This involves biochemical and biophysical mechanisms at the cellular level, i.e. accumulation of sugars (hexoses and sucrose) for osmoregulation controlling freeze dehydration and as cryoprotectants and compatible solutes, where synthesis of glycine betaine also is involved, and regulation of supercooling and extracellular nucleation of ice preventing destruction by formation of intercellular ice crystals (Goldstein and Nobel, 1991, 1994).

The essential component of CAM, i.e. massive nocturnal synthesis of organic acids (phase I), requires high metabolic activity, membrane transport and compartmentation during the night. Thus, while *C<sub>3</sub>* and *C<sub>4</sub>* plants may decrease their metabolic activity during cold nights, seasonal acclimation to low temperatures, which often occur during cold winter nights in deserts, may pose a problem for CAM plants. An interesting observation in this respect was made with *Agave vilmoriniana*, which at low temperatures had higher daytime CO<sub>2</sub> uptake than at high temperatures. Under a day/night temperature regime of 20/10 °C, 92 % of total carbon was taken up during the day but under a regime of 40/30 °C, only 36 % (Nobel and McDaniel, 1988). Hence, this species shifts to a more *C<sub>3</sub>*-like behaviour when temperatures are low, similar to *K. daigremontiana*, which performs *C<sub>3</sub>* photosynthesis at 7 °C (Kluge, 1969). When relatively low day/night temperature regimes change seasonally with higher temperatures, a more *C<sub>3</sub>*-like behaviour may be a good temporal response of obligate CAM plants. The problem is more intriguing at tropical high altitudes, where obligate CAM plants are under low sub-zero temperatures

during each night and high temperatures during each day, without any seasonal changes.

**Salinas.** A number of CAM plants occur in tropical salinas inland or near the coast. The columnar cacti *Stetsonia coryne* and *Cereus validus* are characteristic of the rim of the Salinas Grandes in Argentina (Ellenberg, 1981; Yensen *et al.*, 1981). On alluvial sand plains at the Caribbean coast in Northern Venezuela, the vegetation in salinas is subject to strong seasonal changes, where the sand plains are flooded by fresh water in the rainy season, and covered by a solid crust of salt in the dry season (Medina *et al.*, 1989). However, all CAM plants of this ecosystem are salinity stress avoiders. The columnar cactus *Pilosocereus ottonis* is a salt excluder at the root level (Lüttge *et al.*, 1989), the CAM bromeliad *Tillandsia flexuosa* and the CAM orchid *Schomburgkia humboldtiana* are epiphytes not affected by the salt, and the terrestrial bromeliad *Bromelia humilis* does not produce absorptive soil roots but has tanks and tank roots, fed by rain, instead (Lee *et al.*, 1989). Thus, notwithstanding the observation that CAM plants may determine the physiognomy of salinas, e.g. the large columnar cacti and often a dense ground cover by *B. humilis* on vegetation islands in the salinas, the CAM flora is not really halophytic. Of course, under the various stresses of the habitat the CAM mode of photosynthesis in several ways supports the performance of these plants, which, as salt excluders overcome periods of stress by CAM idling or which are terrestrial and epiphytic life forms being exclusively dependent on water and nutrient input via the atmosphere. However, these adaptations are not specifically salinity related.

**Savannas.** CAM plants are found in tropical savannas and also occur in mesic climate prairies, e.g. cacti and bromeliads in North America (Teeri *et al.*, 1978). However, savannas are dominated by grasses with C<sub>3</sub>, and typically often C<sub>4</sub>, photosynthesis, and by C<sub>3</sub> trees and shrubs. They are not typical CAM habitats. CAM succulents have problems getting established, as they are often overgrown by grasses (Ellenberg, 1981). CAM does not appear to be a particularly suitable adaptation to the seasonally strongly varying precipitation and frequent fires in savannas.

The performance of the obligate C<sub>3</sub> species *Clusia multiflora* and the C<sub>3</sub>/CAM species *C. minor* in a secondary savanna in northern Venezuela showed that *C. multiflora* seemed to be restricted to open, sun-exposed sites. Conversely, *C. minor* mainly occurred in semi-shaded, deciduous forest sites, although it did intrude into the open sites of *C. multiflora* where both species were seen to grow side by side in the same *Clusia* shrubbery (Herzog *et al.*, 1999). *Clusia multiflora* can apparently adapt well to high irradiance during growth. However, when grown at low irradiance and transferred to high irradiance its leaves become necrotic and die, although it can grow new leaves from buds on its stems and become adapted to the new conditions of irradiance. Such photodestruction upon transfer from low to high irradiance was not observed in

*C. minor*. Both species have the xanthophyll cycle complement for harmless dissipation of energy as heat to protect them from photodestruction. In addition, the C<sub>3</sub>/CAM intermediate species *C. minor* can use CAM and its phase III-augmented  $p^i_{CO_2}$  for protection against destructive photoinhibition (Herzog *et al.*, 1999). In this case, it is not CAM *per se* which gives an advantage, but rather the flexibility of C<sub>3</sub>-CAM shifts that increases the niche width of *C. minor*, which is important for its establishment in the habitat. This is corroborated by studies of CAM to C<sub>3</sub> shifts of *C. minor*, which suggest that at high irradiance, and provided water is available, the C<sub>3</sub> mode of photosynthesis is superior to the CAM mode (de Mattos and Lüttge, 2001).

**Restingas.** Restingas are marine sandy deposits and dunes of quaternary origin on the Brazilian coast. Near Rio de Janeiro they date back to the Holocene, 3000–5000 BC, and somewhat further north to the Pleistocene, 120 000 BC. Sub-sites in this ecosystem are sand-dune beach ridges parallel to the coastline with semi-deciduous dry forest in-between and swamp forest around freshwater lagoons where the groundwater table reaches the surface. Restingas are ecosystems of the Atlantic rainforest complex peripheral to the Atlantic rainforest itself on tertiary grounds. They contain very few endemic plant species. The flora is composed of ecologically plastic species that were successful migrants from the Atlantic forest (Scarano, 2002). Restingas have a high species diversity of CAM plants, which is overwhelming at some sites that contain terrestrial and epiphytic bromeliads, cacti and, among the cacti, several epiphytic species, orchids, Piperaceae and *Clusiaceae*. The flexibility of CAM and its associated niche width may have made CAM plants particularly suited to invade these sandy, and often very dry, habitats. In the Atlantic rainforest periphery, most nurse plant species, which protect the establishment of other plants, are either CAM species or belong to families where CAM is present (Scarano, 2002).

On the sand-dune beach ridges, the establishment of vegetation starts in the form of a mosaic of vegetation islands where frequently *Clusia* species are the pioneers or nurse plants under which other vegetation can develop. Thus, Ule (1901) referred to restingas as ‘*Clusia* scrubs’ (Scarano, 2002; Scarano *et al.*, 2004). *Clusia hilariana* and *C. fluminensis* are CAM species that are dominant nurse shrubs and small trees in restingas (Zaluar and Scarano, 2000; Liebig *et al.*, 2001). There are interspecific differences in their ecophysiological performance as shown by comparative studies along a seashore–inland transect with a sand-dune beach ridge, followed by a dry forest and a first sand-dune beach ridge, and by a comparison between a dry restinga and a wet restinga on swampy, sometimes inundated, ground. *Clusia hilariana* was more dominant and overall performed better ecophysiological compared with *C. fluminensis*, which appeared to be limited to the drier sites. Both species showed superior performance compared with that of the C<sub>3</sub> *C. parviflora* on a nearby inselberg (Scarano *et al.*, 2004). Thus, although C<sub>3</sub> vegetation is also abundant, restingas are one of the most unique CAM domains known (Reinert *et al.*, 1997).

**Forests.** Tropical forests appear to be the strongest CAM domains of all habitats.

Semi-deciduous, deciduous and scrub forests may have a dense ground cover of CAM plants, e.g. *Bromelia humilis* together with abundant epiphytic CAM bromeliads in a tropical dry forest in Venezuela (Lüttge, 1997). An ecophysiological study of bromeliads in Trinidad also included terrestrial CAM bromeliads in a dry forest (Plant, Cell and Environment, 1986). Terrestrial CAM plants are also found in moist tropical forests. There, however, the epiphytes constitute the most important species diversity and biomass of CAM plants. It was estimated that about 10 % of all vascular plants are epiphytes, i.e. approx. 23 500 species (Lüttge, 1989b). Of these vascular epiphytes, 57 %, i.e. 13 400 species, are CAM plants (Lüttge, 2003b). In some wet tropical forests 50 % of all leaf biomass may be due to epiphytes or close to 30 % to CAM plants, depending on the abundance of CAM plants among epiphytes.

Due to the general interest in epiphytes (Lüttge, 1989a, 1997; Benzing, 1990, 2000), much is known about the ecology and ecophysiology of CAM epiphytes. They share with C<sub>3</sub> epiphytes many morphotypic and physiotypic traits which adapt them to the problems of water and nutrient availability and the specific light climate of the epiphytic habitat, and morphotypically very similar C<sub>3</sub> and CAM epiphytes can often be found growing side by side on branches of their phorophyte (Griffiths *et al.*, 1986). However, CAM is so important among epiphytes that it must be considered as a central element of epiphyte ecophysiology syn-ecologically at the community level.

In Trinidad, the relation of epiphytic CAM bromeliads to total epiphytic bromeliads decreases from 100 % in dry deciduous forest to less than 10 % in very wet sub-alpine fog or cloud forest (Smith, 1989). However, in an extremely wet cloud forest of Panamá, epiphytic CAM bromeliads of the genus *Aechmea* seem to possess a number of ecophysiological advantages over C<sub>3</sub> bromeliads (Pierce *et al.*, 2002). The flexibility of CAM in CO<sub>2</sub> acquisition allows superior carbon gain and carbon budgets when leaves are frequently wetted, i.e. re-circulation of respiratory CO<sub>2</sub> during the dark period and flexible expression of CAM phases. It is possible that these CAM species only recently intruded into the moist forest habitat. The scarcity of CAM taxa in cloud forests suggests that it is a recent radiation of this photosynthetic pathway and occupation of new niches where the plasticity inherent in CAM is pre-adapting more xerophytic physiotypes to wetter habitats.

**Inselbergs.** Inselbergs are rock outcrops, several tens to several hundreds of metres high, emerging from savannas or rain forests (Lüttge, 1997; Porembski and Barthlott, 2000). Their flora is often highly endemic. They are strongly fragmented into a variety of smaller ecological units, such as humus accumulating in cracks and hollows, vegetation islands, shrubberies and even small forests, especially on their tops. Many CAM families, i.e. Cactaceae, Crassulaceae, Euphorbiaceae, Agavaceae, Bromeliaceae and Orchidaceae, are frequently found on inselbergs in the palaeotropics and the neotropics (Kluge and Brulfert, 2000).

CAM *Clusiaceae* also form small and large scrubs on inselbergs in the neotropics (Scarano *et al.*, 2004). The ecophysiology of CAM bromeliads, including wild species of *Ananas*, and of *Clusia* has been studied on an inselberg in the medium Orinoco basin, Venezuela (Lüttge, 1997). *Clusia* has also been studied on an inselberg in Brazil (Scarano *et al.*, 2004). On an inselberg in Madagascar, the distribution of three *Kalanchoë* species with different expression of CAM was found to be related to micro-habitat characteristics: *K. campanulata* with a very weak CAM capacity in the shade of deep humid gaps performed largely C<sub>3</sub> photosynthesis, *K. miniata* in open bush formations showed pronounced CAM performance, while *K. synsepala* spreading by stolons on the bare rock showed the strongest expression of CAM (Kluge and Brulfert, 2000).

**High altitudes.** The earliest study of the relationship between altitude and CAM was performed in the European Alps (Osmond *et al.*, 1975) based mainly on a survey of <sup>13</sup>C-discrimination. Four alpine *Sempervivum* species were shown to be CAM plants, and three alpine *Sedum* species and other succulents showed C<sub>3</sub> behaviour. Bachereau *et al.* (1998) suggest that for *Sedum album*, synthesis of phenolics as UV-protectants is important at higher altitudes in the Alps. Accumulation of phenolics and expression of CAM (CAM cycling in this case) are inversely related, so that at higher altitudes CAM performance occurs only in the shade. Temperature may be the most important factor limiting CAM at higher altitudes. For *Sempervivum montanum*, Wagner and Larcher (1981) showed that daytime remobilization of malic acid was reduced in an approximately linear way from 40 °C down to 15 °C. A survey of orchids in Papua New Guinea, based on analyses of <sup>13</sup>C-discrimination, suggested a gradual decline of CAM among species at increasing altitude. In a lowland rainforest at 200–300 m a.s.l., 26 % of species were obligate and another 25 % possibly weak facultative CAM species, while in a lower montane forest at 1175 m a.s.l., 26 % of the orchids were obligate CAM species and there were apparently no weak CAM performers. In an upper montane and a sub-alpine forest at 2600–3600 m a.s.l. CAM was lacking (Earnshaw *et al.*, 1987). Among *Clusiaceae* in the coastal cordillera of Venezuela, CAM is restricted to ≤1500 m a.s.l. (Díaz *et al.*, 1996).

In relation to temperature, the occurrence of CAM plants at very high altitudes in tropical mountains, especially the paramos of South America, is intriguing. There are cacti in the Andes (Keeley and Keeley, 1989) and the Crassulaceae *Echeveria columbiana* occurs in Venezuela (Medina and Delgado, 1976). These high altitudes at 3500 m a.s.l. and above are characterized by a regular diurnal climate change with ‘summer every day and winter every night’ (Hedberg, 1964). Although C<sub>3</sub> plants with specific adaptations dominate the vegetation, there are obligate CAM plants, and it would be interesting to know how they manage a metabolism of nocturnal organic acid accumulation at sub-freezing temperatures every night. A long-term acclimation, as in lowland deserts and prairies in North America where nocturnal frost is seasonally restricted to the winter, would

not help in the diurnal frost climate of the paramós. There appears to be no research on this problem.

## CONCLUSIONS

Within plant types, which are the *receivers* of the environmental *input* and modulate it to an *output* of community formation, there are, with the exception of leaf and stem succulence, no specialities of particular life forms or morphotypes of CAM plants. Among the terrestrial CAM habitats in the subtropics and tropics, salinas, savannas and paramós do not accommodate major cohorts of CAM plants. Among the smaller ecosystems, some restingsas and inselbergs appear to be very strong domains of CAM. Regarding species diversity, the largest CAM territories are tropical dry forests and the various types of tropical wet forests and rainforests. The latter are even more important CAM habitats than deserts (Table 3). However, with respect to vegetation cover, some restingsas may be the most conspicuous CAM habitats.

The stronger CAM domains all appear to have one thing in common, namely that environmental stress is caused by the action of a network of different factors and not by one single dominant factor (Fig. 1). With a multifactor network of environmental stresses acting in the multitude of ecological niches of restingsas and inselbergs and, particularly, tropical forests it appears that it is the flexibility of CAM and the associated large phenotypic plasticity of CAM plants that constitute the advantage of CAM for acquisition of ecological niches.

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